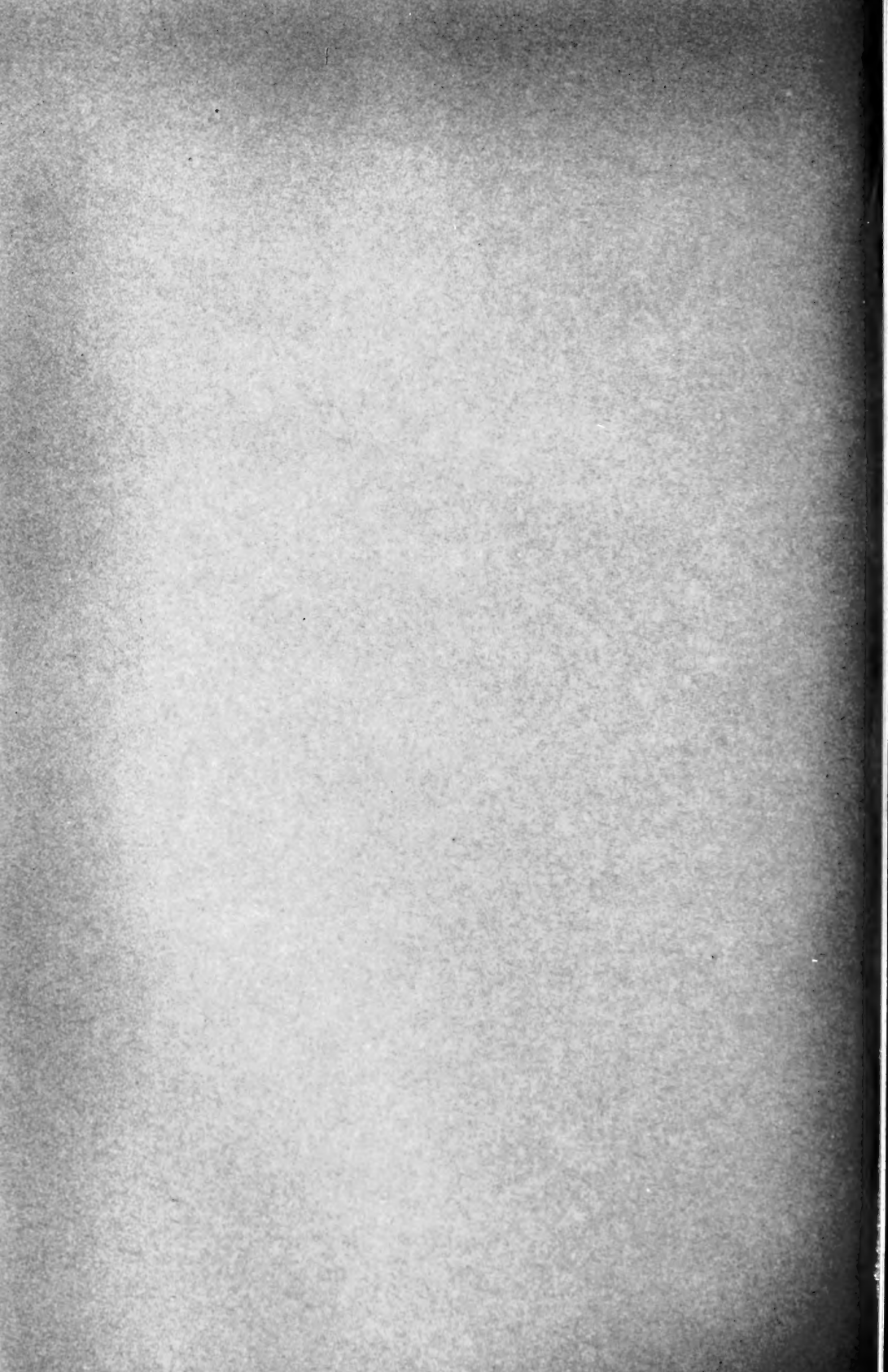


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AGRICULTURAL EXPERIMENT STATION
OF
NEBRASKA

STUDIES OF IMMUNITY AGAINST HEMOR-
RHAGIC SEPTICEMIA

BY L. VAN ES AND H. M. MARTIN
DEPARTMENT OF ANIMAL PATHOLOGY AND HYGIENE

AUGUST, 1922

LINCOLN, NEBRASKA
U. S. A.

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STUDIES OF IMMUNITY AGAINST HEMORRHAGIC SEPTICEMIA

L. VAN ES AND H. M. MARTIN

DEPARTMENT OF ANIMAL PATHOLOGY AND HYGIENE

Within recent years a considerable interest has been developed in the part played by *Bacillus bipolaris septicus* in the production of disease among farm live stock. Hemorrhagic septicemia was and is yet represented to be a prolific source of loss among the domesticated mammals. So far as our own observations pertaining to Nebraska live stock are concerned, there does not seem to be cause to attach great importance to this disease as a menace to cattle, sheep, and swine. In our investigations we very rarely encountered this infection, in spite of considerable effort to do so and in spite of an abundance of suspected material from cattle, sheep, and swine.

In only one direction can we recognize the organism as a serious source of loss, and that is in connection with fowl cholera, a disease which exacts a heavy annual toll from our poultry raisers.

It is this last named disease above all which gives warrant for a study of the immunology of hemorrhagic septicemia in general.

As one of the phases of our inquiry an effort was made to acquire some general knowledge regarding the immunizing value (1) of sera prepared with the aid of *Bacillus bipolaris septicus* procured from cattle and swine or (2) of those alleged to be of service in the control or treatment of hemorrhagic septicemia in the species mentioned. The experiments reported in this publication were almost exclusively made with sera purchased from dealers in biologic products.

The sera examined were prepared by means of strains of *Bacillus bipolaris septicus* obtained from cattle and swine or which were represented, at least, to be useful in the management of hemorrhagic septicemia in the species named.

In the test of the sera (1) represented to be useful in the control of the disease in cattle or (2) which were prepared by means of a cattle strain of the *Bacillus bipolaris septicus*, use was made of cultures of our cattle strain No. 620, while in

the series (1) pertaining to the sera intended to be used in swine or (2) made with the aid of strains derived from swine, our swine strain No. 59 was employed. The virus used always consisted of bouillon cultures at the end of a twenty-four hour incubation.

Rabbits were used as test animals. The serum was injected subcutaneously, while the virus was injected intravenously. The intravenous virus injections were preferred after previous experiments had shown that in this manner the annoying formation of abscesses and necrotic areas could be avoided, while the difference between the results obtained with intravenous virus injections and subcutaneous ones was only expressed by a slightly shorter incubation period in the case of the former.

For so far as this was possible each serum was utilized in two test series. In one of them there was variation in the doses of serum given. All the animals in this series received the serum on a given day, while the virus was injected twenty-four hours later. In the other series all the rabbits received ten c.c. of serum on a given day. The series was then divided into four or five groups, each of which received the required amount of virus at periods from three to five days apart.

In the first series the influence of the amounts of serum was taken into consideration, while in the latter we aimed to obtain information regarding the duration of the passive immunity, in case any should be manifest at all.

Second injections of virus were given to all surviving animals, usually some two weeks after the first virus injection was administered, with a view of ascertaining whether or not the subsequent injection of virus into a serum protected animal would engender a more enduring and active immunity.

BOVINE STRAIN SERA

The first series comprises the tests made with varying doses of serum. In one of the tests (No. 1052) a parallel number of rabbits were injected with normal horse serum in order to properly appraise whatever nonspecific protective influence may be attached to a foreign serum per se.

The details of the tests are exhibited in Tables 1-11.

TABLE 1.

1921

No. 1052

Rabbit No.	Injection of serum No. 1052, Feb. 11, c.c.	Injection of virus No. 620, Feb. 12, 1/500 c.c.	Injection of virus No. 620, Feb. 27, 1/600 c.c.	Dates of deaths	Remarks
1	15	+	+	March 1	
2	15	+	+	Feb. 28	
3	15	+	+	Feb. 28	
4	10	+	+	March 1	
5	10	+	+	March 2	
6	10	+	+	March 1	
7	5	+	+	March 1	
8	5	+	+	March 1	
9	5	+	—	Feb. 18	
10	2.5	+	—	Feb. 17	
11	2.5	+	+	March 2	
12	2.5	+	—	Feb. 18	

NOTE—The fact that an injection was made is indicated by the +. A dash (—) is used to show that no injection took place.

TABLE 2.

1921

(Normal) No. 1052

Rabbit No.	Injection of normal serum, Feb. 11, c.c.	Injection of virus No. 620, Feb. 12, 1/500 c.c.	Injection of virus No. 620, Feb. 27, 1/600 c.c.	Dates of deaths	Remarks
1	15	+	—	Feb. 17	
2	15	+	+	March 7	
3	15	+	—	Feb. 14	
4	10	+	—	Feb. 14	
5	10	+	—	Feb. 15	
6	10	+	—	Feb. 14	
7	5	+	—	Feb. 14	
8	5	+	—	Feb. 14	
9	5	+	—	Feb. 15	
10	2.5	+	—	Feb. 15	
11	2.5	+	—	Feb. 14	
12	2.5	+	+	?	Record of date of death lost.

1921

TABLE 3.

No. 1052

Rabbit No.	Injection of virus No. 620, Feb. 12, 1/500 c.c.	Dates of deaths	Remarks
1	+	Feb. 14	} Virus control (first injections)
2	+	Feb. 14	
3	+	Feb. 14	

1921

TABLE 4.

No. 1052

Rabbit No.	Injection of virus No. 620, Feb. 27, 1/600 c.c.	Dates of deaths	Remarks
1	+	Feb. 28	} Virus control (second injections)
2	+	Mch. 1	

1921

TABLE 5.

No. 1178

Rabbit No.	Injection of serum No. 1178, April 11, c.c.	Injection of virus No. 620, April 12, 1/500 c.c.	Injection of virus No. 620, April 26, 1/600 c.c.	Dates of deaths	Remarks
1	15	+	+	Apr. 27	B. bipolaris swarming in blood. Pericarditis. Necrotic pneumonia. Empyema. Severe pneumonia. Heart blood swarming with B. bipolaris. Pneumonia. Heart blood swarming with B. bipolaris.
2	15	+	—	Apr. 19	
3	15	+	—	Apr. 17	
4	15	+	—	Apr. 20	
5	10	+	+	Apr. 30	Few B. bipolaris in heart blood. Fibrinous pericarditis. Pneumonia. Abscess in lung. Blood swarming with B. bipolaris.
6	10	+	—	Apr. 18	
7	10	+	+	Apr. 28	
8	10	+	—	Apr. 16	
9	5	+	+	Apr. 27	Control 1st test.
10	5	+	+	Apr. 27	
11	5	+	+	Apr. 28	
12	5	+	+	Apr. 28	
13	—	+	—	Apr. 13	Control 1st test.
14	—	+	—	Apr. 14	Control 1st test.
15	—	+	—	Apr. 14	Control 1st test.
16	—	+	+	Apr. 28	Control 1st test.
17	—	—	+	—	Control 2nd test. Lives.
18	—	—	+	Apr. 28	Control 2nd test.

1922

TABLE 6.

No. 1178

Rabbit No.	Injection of serum No. 1178, Feb. 6, c.c.	Injection of virus No. 620, Feb. 7, 1/500 c.c.	Injection of virus No. 620, Feb. 22, 1/500 c.c.	Dates of deaths	Remarks
1	5	+	+	Feb. 25	Heart blood swarming with <i>B. bipolaris</i> .
2	5	+	+	Feb. 25	
3	5	+	—	Feb. 12	
4	10	+	—	Feb. 13	
5	10	+	—	Feb. 15	
6	10	+	—	Feb. 15	
7	15	+	+	Feb. 25	
8	15	+	—	Feb. 15	
9	15	+	+	March 6	

1922

TABLE 7.

No. 1434

Rabbit No.	Injection of serum No. 1434, Feb. 6, c.c.	Injection of virus No. 620, Feb. 7, 1/500 c.c.	Injection of virus No. 620, Feb. 22, c.c.	Dates of deaths	Remarks
1	5	+	—	Feb. 13	
2	5	+	—	Feb. 13	
3	5	+	—	Feb. 12	
4	10	+	—	Feb. 13	
5	10	+	+	Feb. 24	
6	10	+	—	Feb. 14	
7	15	+	+	Feb. 24	
8	15	+	+	Feb. 24	
9	15	+	+	Feb. 25	

1922

TABLE 8.

No. 1828

Rabbit No.	Injection of serum No. 1828, Feb. 6, c.c.	Injection of virus No. 620, Feb. 7, 1/500 c.c.	Injection of virus No. 620, Feb. 22, 1/500 c.c.	Dates of deaths	Remarks
1	5	+	—	Feb. 14	
2	5	+	—	Feb. 14	
3	5	+	—	Feb. 13	
4	10	+	—	Feb. 19	
5	10	+	+	Feb. 23	
6	10	+	—	Feb. 14	
7	15	+	+	Feb. 23	
8	15	+	—	Feb. 16	
9	15	+	—	Feb. 15	

1922

TABLE 9.

No. 1829

Rabbit No.	Injection of serum No. 1829, Feb. 6, c.c.	Injection of virus No. 620 Feb. 7, 1/500 c.c.	Dates of deaths	Remarks
1	5	+	Feb. 10	
2	5	+	Feb. 10	
3	5	+	Feb. 9	
4	10	+	Feb. 13	
5	10	+	Feb. 12	
6	10	+	Feb. 11	
7	15	+	Feb. 11	
8	15	+	Feb. 12	
9	15	+	Feb. 14	

1922

TABLE 10.

No. 1830

Rabbit No.	Injection of serum No. 1830, Feb. 6, c.c.	Injection of virus No. 620, Feb. 7, 1/500 c.c.	Dates of deaths	Remarks
1	5	+	Feb. 10	
2	5	+	Feb. 10	
3	5	+	Feb. 8	
4	10	+	Feb. 8	
5	10	+	Feb. 13	
6	10	+	Feb. 10	
7	15	+	Feb. 13	
8	15	+	Feb. 11	
9	15	+	Feb. 10	

TABLE 11.

1922

Controls 1178—1434—1828—1829—1830

Rabbit No.	Injection of virus No. 620, Feb. 7, 1/500 c.c.	Dates of deaths	Remarks
1	+	Feb. 8	
2	+	Feb. 8	
3	+	Feb. 8	
4	+	Feb. 8	
5	+	Feb. 8	
6	+	Feb. 8	
7	+	Feb. 8	
8	+	Feb. 8	
9	+	Feb. 8	
10	+	Feb. 8	

The results of the preceding tests are summarized in Tables 12 and 13 and they show that the protective influence of the serum was quite manifest, altho it does not appear that the variation in the doses as used in the series was very strikingly shown in the totals. There were differences, but they were neither conspicuous nor consistent. There appears, however, to be a marked difference in the potency of some of the sera examined. The tables show that protective influence of the sera examined manifested itself in two ways,—in the first place by actually preventing the death of experimental animals when injected with virulent cultures, and in the second place by increasing the approximate surviving period, after virus inoculation, of the serum-treated animals which actually succumbed, as compared with the ones treated with normal serum and with those which served as virus controls.

Of the 69 rabbits treated with serum and virus, 26 survived. Approximately two weeks after the latter had received the first virus injection, they were inoculated again with the same virus.

Table No. 14 shows that none of those survivors had become resistant to infection as a result of the serum-virus treatment they had previously received. A certain lengthening of the surviving periods after the last virus injection could be observed. (See Table 15.)

TABLE 12.

Results of virus injections made one day after serum injected in the following doses.

Serum No.	15 c.c.		10 c.c.		5 c.c.		2.5 c.c.		All doses	
	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
1052	0	3	0	3	1	2	2	1	3	9
1178	3	1	2	2	0	4	—	—	5	7
1178	1	2	3	0	1	2	—	—	5	4
1434	0	3	2	1	3	0	—	—	5	4
1828	2	1	2	1	3	0	—	—	7	2
1829	3	0	3	0	3	0	—	—	9	0
1830	3	0	3	0	3	0	—	—	9	0
Totals	12	10	15	7	14	8	2	1	43	26

Normal 1052...	2	1	3	0	3	0	2	1	10	2
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Results of virus injections without preceding serum injections.

[illegible]

TABLE 13.

Lengths of surviving periods of animals which died on account of virus injections on the day following serum treatment.

	Approximate averages of surviving periods by hours									
	15 c.c.		10 c.c.		5 c.c.		2.5 c.c.		All doses	
Serum No.	No.of rabbits	Surviving periods	No.of rabbits	Surviving periods	No.of rabbits	Surviving periods	No.of rabbits	Surviving periods	No.of rabbits	Surviving periods
1052	—	—	—	—	1	168	2	156	3	160
1178	1	192	3	176	1	120	—	—	5	168
1178	3	160	2	120	—	—	—	—	5	144
1434	—	—	2	156	3	136	—	—	5	144
1828	2	204	2	228	3	160	—	—	7	192
1829	3	128	3	120	3	64	—	—	9	104
1830	3	104	3	80	3	56	—	—	9	80
Totals and av'gs	12	148	15	142	14	109	2	156	43	134

Normal 1052.....	2	84	3	56	3	56	—	—	8	63
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Results of virus injections in animals not treated with serum.

[illegible]

TABLE 14.

Results of second virus injections in serum-virus treated rabbits.

[illegible]

TABLE 15.

Lengths of surviving periods of animals which were serum-virus treated and which died as a result of a second virus injection.

Serum No.	Approximate averages of surviving periods by doses and hours							
	15 c.c.		10 c.c.		5 c.c.		All doses	
	No. of rabbits	Surviving periods	No. of rabbits	Surviving periods	No. of rabbits	Surviving periods	No. of rabbits	Surviving periods
1052.....	3	32	3	56	2	48	8	45
1178.....	1	24	2	72	4	36	7	44 +
1178.....	2	180	—	—	2	72	4	126
1434.....	3	56	1	48	—	—	4	54
1828.....	1	24	1	24	—	—	2	24
Totals and averages..	10	67	7	55	8	48	25	57
Virus control..	—	—	—	—	—	—	8	30

Tables Nos. 16-23 exhibit the details of the series in which a uniform dose of serum was given and the latter followed by the virus inoculation of the various groups at times further and further removed from the day of serum treatment.

The results of the experiments set forth in Tables 16-23 are summarized in Table 24. They indicate that the animals used in the experiment survived only exceptionally when the virus dose was given from one to several days after the serum injections.

The influence of the serum in the series is only shown by the lengthening of the surviving periods of the treated rabbits. This phenomenon is shown in Table 25. In comparison with the virus-control rabbits, there is a marked tendency on the part of the serum-treated animals to survive longer after the virus inoculations. The differences gradually disappear as the periods elapsing between the injections of the serum and those of the virus become longer. This behavior is shown by

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TABLE 16.

No. 1052

Rabbit No.	Injection of serum No. 1052, March 17, c.c.	Injection of virus No. 620, 1/500 c.c. Dates	Dates of deaths	Remarks
1	10	Mch. 18	Mch. 28	Heart blood swarming with <i>B. bipolaris</i> .
2	10	Mch. 18	Mch. 25	
3	—	Mch. 18	Mch. 20	Control.
4	—	Mch. 18	Mch. 20	Control.
5	10	Mch. 21	Mch. 27	Few organisms in heart blood, but abscess at point of inoculation swarming with same.
6	10	Mch. 21	Mch. 27	Very few <i>B. bipolaris</i> in blood.
7	—	Mch. 21	Mch. 22	Control.
8	—	Mch. 21	Mch. 23	Control.
9	10	Mch. 25	April 1	<i>B. bipolaris</i> in blood.
10	10	Mch. 25	Mch. 29	Few <i>B. bipolaris</i> in blood.
11	—	Mch. 25	Mch. 28	Control.
12	—	Mch. 25	Mch. 27	Control.
13	10	Mch. 28	Mch. 31	<i>B. bipolaris</i> in blood.
14	10	Mch. 28	Mch. 31	<i>B. bipolaris</i> in blood.
15	—	Mch. 28	Mch. 29	Control.
16	—	Mch. 28	Mch. 30	Control.
17	10	Mch. 31	April 5	Numerous <i>B. bipolaris</i> in blood.
18	10	Mch. 31	April 2	<i>B. bipolaris</i> in blood.
19	—	Mch. 31	April 2	Control.
20	—	Mch. 31	April 2	Control.

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TABLE 17.

No. 1440

Rabbit No.	Injection of serum No. 1440, Jan. 16, 10 c.c.	Injection of virus No. 620, 1/500 c.c.						Dates of deaths	Remarks
		8/19	8/23	8/27	8/31	9/3			
1	+	+	—	—	—	—		Aug. 25	
2	+	+	—	—	—	—		Aug. 23	
3	—	+	—	—	—	—		Aug. 20	
4	—	+	—	—	—	—		Aug. 20	
5	+	—	+	—	—	—		Aug. 25	
6	+	—	+	—	—	—		Aug. 25	
7	—	—	+	—	—	—		Aug. 25	
8	—	—	+	—	—	—		Aug. 26	
9	+	—	—	+	—	—		Aug. 28	
10	+	—	—	+	—	—		Aug. 28	
11	—	—	—	+	—	—		Aug. 28	
12	—	—	—	+	—	—		Aug. 28	
13	+	—	—	—	+	—		Sept. 2	
14	+	—	—	—	+	—		Sept. 1	
15	—	—	—	—	+	—		Sept. 1	
16	—	—	—	—	+	—		Sept. 1	
17	+	—	—	—	—	+		Discharged healthy, 9/15.
18	—	—	—	—	—	+		Discharged healthy, 9/19.

TABLE 18.

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No. 1522

Rabbit No.	Injection of serum No. 1522, Aug. 16, 10 c.c.	Injections of virus No. 620, 1/500 c.c.		Dates of deaths	Remarks
		8/23	8/27		
1	+	+	—	Aug. 25	
2	+	+	—	Aug. 25	
3	+	+	—	Aug. 25	
4	—	+	—	Aug. 25	
5	—	+	—	Aug. 26	
6	+	—	+	Aug. 28	
7	+	—	+	Aug. 28	
8	—	—	+	Aug. 28	
9	—	—	+	Aug. 28	

TABLE 19.

1922

No. 1178

Rabbit No.	Injection of serum No. 1178, Feb. 6, 10 c.c.	Injection of virus No. 620, 1/500 c.c.				Dates of deaths	Remarks
		2/10	2/14	2/18	2/22		
1	+	+	—	—	—	Feb. 14	
2	+	+	—	—	—	Feb. 17	
3	+	+	—	—	—	Feb. 16	
4	—	+	—	—	—	Feb. 10	
5	—	+	—	—	—	Feb. 11	
6	+	—	+	—	—	Feb. 16	
7	+	—	+	—	—	Feb. 16	
8	+	—	+	—	—	Feb. 15	
9	—	—	+	—	—	Feb. 15	
10	—	—	+	—	—	Feb. 15	
11	+	—	—	+	—	Feb. 19	
12	+	—	—	—	—	Feb. 17	Probable cause of death, abscess of head.
13	+	—	—	+	—	Feb. 19	
14	—	—	—	+	—	Feb. 19	
15	+	—	—	—	+	Feb. 24	
16	+	—	—	—	+	Feb. 23	
17	+	—	—	—	+	Feb. 24	
18	—	—	—	—	+	Feb. 23	

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TABLE 20.

No. 1434

Rabbit No.	Injection of serum No. 1434, Feb. 6, 10 c.c.	Injection of virus No. 620, 1/500 c.c.				Dates of deaths	Remarks
		2/10	2/14	2/18	2/22		
1	+	+	—	—	—	Feb. 14	
2	+	+	—	—	—	Feb. 13	
3	+	+	—	—	+	Feb. 23	
4	—	+	—	—	—	Feb. 11	
5	—	+	—	—	—	Feb. 11	
6	+	—	+	—	—	Feb. 17	
7	+	—	+	—	—	Feb. 16	
8	+	—	+	—	—	Feb. 16	
9	—	—	+	—	—	Feb. 15	
10	—	—	+	—	—	Feb. 15	
11	+	—	—	+	—	Feb. 19	
12	+	—	—	+	—	Feb. 19	
13	+	—	—	+	+	Feb. 25	
14	—	—	—	+	—	Feb. 19	
15	+	—	—	—	+	Feb. 23	
16	+	—	—	—	+	Feb. 23	
17	+	—	—	—	+	Feb. 23	
18	—	—	—	—	+	Feb. 23	

1922

TABLE 21.

No. 1828

Rabbit No.	Injection of serum No. 1828, Feb. 6, 10 c.c.	Injection of virus No. 620, 1/500 c.c.				Dates of deaths	Remarks
		2/10	2/14	2/18	2/22		
1	+	+	—	—	—	Feb. 17	
2	+	+	—	—	—	Feb. 17	
3	+	+	—	—	—	Feb. 14	
4	—	+	—	—	—	Feb. 11	
5	—	+	—	—	—	Feb. 11	
6	+	—	+	—	—	Feb. 15	
7	+	—	+	—	—	Feb. 16	
8	+	—	+	—	—	Feb. 15	
9	—	—	+	—	—	Feb. 15	
10	—	—	+	—	—	Feb. 15	
11	+	—	—	+	—	Feb. 19	
12	+	—	—	+	—	Feb. 19	
13	+	—	—	+	—	Feb. 19	
14	—	—	—	+	—	Feb. 19	
15	+	—	—	—	+	Feb. 23	
16	+	—	—	—	+	Feb. 24	
17	+	—	—	—	+	Feb. 23	
18	—	—	—	—	+	Feb. 23	

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TABLE 22.

No. 1829

Rabbit No.	Injection of serum No. 1829, Feb. 6, 10 c.c.	Injection of virus No. 620, 1/500 c.c.				Dates of deaths	Remarks
		2/10	2/14	2/18	2/22		
1	+	+	—	—	—	Feb. 11	
2	+	+	—	—	—	Feb. 11	
3	+	+	—	—	—	Feb. 12	
4	—	+	—	—	—	Feb. 11	
5	—	+	—	—	—	Feb. 11	
6	+	—	+	—	—	Feb. 15	
7	+	—	+	—	—	Feb. 15	
8	+	—	+	—	—	Feb. 15	
9	—	—	+	—	—	Feb. 15	
10	—	—	+	—	—	Feb. 15	
11	+	—	—	+	—	Feb. 19	
12	+	—	—	+	—	Feb. 20	
13	+	—	—	+	—	Feb. 19	
14	—	—	—	+	+	Mch. 4	
15	—	—	—	—	+	Feb. 23	
16	+	—	—	—	+	Feb. 23	
17	+	—	—	—	+	Feb. 24	
18	+	—	—	—	+	Feb. 23	

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TABLE 23.

No. 1830

Rabbit No.	Injection of serum No. 1830, Feb. 6, 10 c.c.	Injection of virus No. 620, 1/500 c.c.				Dates of deaths	Remarks
		2/10	2/14	2/18	2/22		
1	+	+	—	—	—	Feb. 13	
2	+	+	—	—	—	Feb. 13	
3	+	+	—	—	—	Feb. 13	
4	—	+	—	—	—	Feb. 11	
5	—	+	—	—	—	Feb. 11	
6	+	—	+	—	—	Feb. 16	
7	+	—	+	—	—	Feb. 15	
8	+	—	+	—	—	Feb. 15	
9	—	—	+	—	—	Feb. 15	
10	—	—	+	—	—	Feb. 15	
11	+	—	—	+	—	Feb. 19	
12	+	—	—	+	—	Feb. 19	
13	+	—	—	+	—	Feb. 19	
14	—	—	—	+	—	Feb. 19	
15	+	—	—	—	+	Feb. 24	
16	+	—	—	—	+	Feb. 23	
17	+	—	—	—	+	Mch. 8	
18	—	—	—	—	+	Feb. 23	

the graph in Table 26, which tends to show that the small degree of protection conferred by the serum has practically disappeared after a lapse of seven or eight days.

TABLE 24.

Results of virus inoculations of serum-treated rabbits after varying periods.

Injection of 10 c.c. of serum No.	Virus injection after serum. Days	Serum-treated rabbits		Virus-control rabbits		Remarks
		Alive	Dead	Alive	Dead	
1052.....	1	0	2	0	2	
	4	0	2	0	2	
	8	0	2	0	2	
	11	0	2	0	2	
	14	0	2	0	2	
1440.....	3	0	2	0	2	
	7	0	2	0	2	
	11	0	2	0	2	
	15	0	2	0	2	
1522.....	18	1	0	1	0	
	7	0	3	0	2	
	11	0	2	0	2	
1178.....	4	0	3	0	2	
	8	0	3	0	2	
	12	0	2	0	1	
	16	0	3	0	1	
1434.....	4	1	2	0	2	
	8	0	3	0	2	
	12	1	2	0	1	
	16	0	3	0	1	
1828.....	4	0	3	0	2	
	8	0	3	0	2	
	12	0	3	0	1	
	16	0	3	0	1	
1829.....	4	0	3	0	2	
	8	0	3	0	2	
	12	0	3	1	0	
	16	0	3	0	1	
1830.....	4	0	3	0	2	
	8	0	3	0	2	
	12	0	3	0	1	
	16	0	3	0	1	

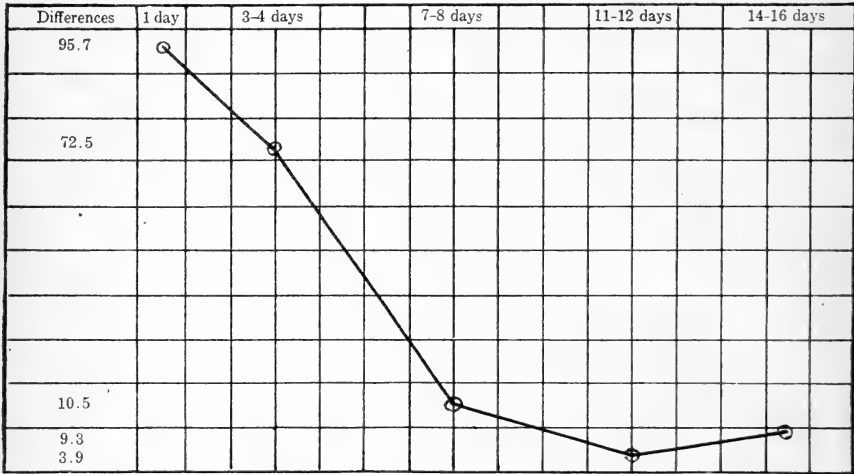
TABLE 25.

The average surviving periods of rabbits dead as the result of virus injections at varying periods after serum treatment, in hours.

Injection of 10 c.c. of serum No.	Virus injection after serum. Days	Serum-treated rabbits		Virus-control rabbits		Remarks
		No. of rabbits	Surviving periods	No. of rabbits	Surviving periods	
1052.....	1	2	204	2	48	
	4	2	144	2	36	
	8	2	132	2	60	
	11	2	72	2	36	
	14	2	84	2	48	
1440.....	3	2	120	2	24	
	7	2	48	2	60	
	11	2	24	2	24	
	15	2	36	2	24	
1522.....	7	3	48	2	36	
	11	2	24	2	24	
1178.....	4	3	136	2	24	
	8	3	40	2	24	
	12	2	24	1	24	
	16	3	40	1	24	
1434.....	4	2	84	2	24	
	8	3	56	2	24	
	12	2	24	1	24	
	16	3	24	1	24	
1828.....	4	3	144	2	24	
	8	3	32	2	24	
	12	3	24	1	24	
	16	3	32	1	24	
1829.....	4	3	32	2	24	
	8	3	24	2	24	
	12	3	32	1	336	
	16	3	32	1	24	
1830.....	4	3	72	2	24	
	8	3	32	2	24	
	12	3	24	1	24	
	16	3	136	1	24	

TABLE 26.

Differences, in hours, between surviving periods of serum-treated rabbits and of corresponding virus-control rabbits at varying periods after serum treatment.



N. B. The evidence of one virus-control rabbit lingering 336 hrs. and of one serum-treated rabbit lingering 408 hrs. was not included in this graph.

SWINE STRAIN SERA

In this series the same general plan of inquiry as reported on the preceding pages was followed. The first part of the tests made pertain to sera which were injected in varying doses. In one of the tests (No. 1053) a control set of rabbits injected with normal serum was used in order to be able to estimate whatever influence a normal serum may have. The details of the serum tests in this series are presented in Tables 27-34.

TABLE 27.

1921

No. 1053

Rabbit No.	Injection of serum No. 1053, Feb. 11, c.c.	Injection of virus No. 59, Feb. 12, 1/5,000 c.c.	Injection of virus No. 59, Feb. 27, 1/6,000 c.c.	Dates of deaths	Remarks
1	15	+	+	Feb. 28	
2	15	+	+	Feb. 28	
3	15	+	+	Mch. 1	
4	10	+	+	Mch. 1	
5	10	+	+	Feb. 28	
6	10	+	+	Feb. 28	
7	5	+	—	Feb. 15	
8	5	+	+	Feb. 28	
9	5	+	+	Feb. 28	
10	2.5	+	+	Feb. 28	
11	2.5	+	+	Mch. 7	
12	2.5	+	+	Mch. 1	

TABLE 28.

1921

(Normal serum) No. 1053

Rabbit No.	Injection of normal serum, Feb. 11, c.c.	Injection of virus No. 59, Feb. 12, 1/5,000 c.c.	Injection of virus No. 59, Feb. 27, 1/6,000 c.c.	Dates of deaths	Remarks
1	15	+	—	Feb. 14	
2	15	+	—	Feb. 14	
3	15	+	—	Feb. 17	
4	10	+	—	Feb. 15	
5	10	+	—	Feb. 15	
6	10	+	—	Feb. 15	
7	5	+	—	Feb. 16	
8	5	+	—	Feb. 14	
9	5	+	—	Feb. 14	
10	2.5	+	—	Feb. 20	
11	2.5	+	—	Feb. 14	
12	2.5	+	—	Feb. 15	

TABLE 29.

1921

No. 1053

Rabbit No.	Injection of virus No. 59, February 12, 1/5,000 c.c.	Dates of deaths	Remarks
1	+	Feb. 18	Virus control (first injection).
2	+	Feb. 14	Virus control (first injection).
3	+	Feb. 14	Virus control (first injection).

TABLE 30.

1921

No. 1053

Rabbit No.	Injection of virus No. 59, February 27, 1/6,000 c.c.	Dates of deaths	Remarks
1	+	Feb. 28	Virus control (second injections).
2	+	Feb. 28	Virus control (second injections).

TABLE 31.

1921

No. 1433

Rabbit No.	Injection of serum No. 1433, Aug. 26, c.c.	Injection of virus No. 59, Aug. 27, 1/5,000 c.c.	Injection of virus No. 59, Sept. 9, 1/6,000 c.c.	Dates of deaths	Remarks
1	5	+	—	Sept. 8	
2	5	+	—	Aug. 29	
3	5	+	—	Sept. 1	
4	10	+	—	Aug. 31	
5	10	+	+	Sept. 10	
6	10	+	—	Sept. 2	
7	15	+	—	Sept. 2	
8	15	+	—	Sept. 4	
9	15	+	—	Sept. 3	
10	—	+	—	Aug. 28	
11	—	+	—	Sept. 1	

TABLE 32.

1921

No. 1436

Rabbit No.	Injection of serum No. 1436, Sept. 9, c.c.	Injection of virus No. 59, Sept. 10, 1/5,000 c.c.	Injection of virus No. 59, Sept. 22, 1/5,000 c.c.	Dates of deaths	Remarks
1	5	+	+	Sept. 23	
2	5	+	—	Sept. 17	
3	5	+	—	Sept. 15	
4	10	+	+	Sept. 23	
5	10	+	—	Sept. 14	
6	10	+	+	Sept. 23	
7	15	+	—	Sept. 20	
8	15	+	+	Sept. 23	
9	15	+	—	Sept. 18	
10	—	+	—	Sept. 11	
11	—	+	—	Sept. 11	
12	—	—	+	Sept. 23	
13	—	—	+	Sept. 23	

TABLE 33.

1922

No. 1945

Rabbit No.	Injection of serum No. 1945, March 13, c.c.	Injection of virus No. 59, March 14, 1/10,000 c.c.	Injection of virus No. 59, March 29, 1/10,000 c.c.	Dates of deaths	Remarks
1	5	+	—	Mar. 15	
2	5	+	—	Mar. 16	
3	5	+	+	
4	10	+	—	Mar. 15	
5	10	+	—	Mar. 15	
6	10	+	—	Mar. 15	
7	15	+	—	Mar. 15	
8	15	+	—	Mar. 15	
9	15	+	—	Mar. 15	
10	—	+	—	Mar. 15	
11	—	+	—	Mar. 15	
12	—	+	—	Mar. 15	

TABLE 34.

1922

No. 1946

Rabbit No.	Injection of serum No. 1946, March 13, c.c.	Injection of virus No. 59, March 14, 1/10,000c.c.	Injection of virus No. 59, March 29, 1/10,000c.c.	Dates of deaths	Remarks
1	5	+	+	Mar. 30	
2	5	+	+	Mar. 30	
3	5	+	+	Mar. 30	
4	10	+	+	
5	10	+	+	Mar. 30	
6	10	+	+	Mar. 30	
7	15	+	+	Mar. 30	
8	15	+	—	Mar. 24	
9	15	+	+	Mar. 30	
10	—	+	—	Mar. 16	
11	—	+	—	Mar. 15	
12	—	+	—	Mar. 15	

The results of the serum tests shown by Tables 27-34 are summarized and exhibited in Tables 35-36. It is shown that the variations in the doses were not followed by corresponding differences in the results obtained. It appears that the range between 5 and 15 c.c. is not great enough to show the influence of quantity. The results also show a wide variation in the protective qualities of the sera experimented with. In some the potency was good, in others it was scarcely above that of normal serum. Of the 48 rabbits used in this series, 25 were actually prevented from succumbing to the infection by the use of the serum.

The influence of the serum is further shown by the longer surviving periods of the serum treated rabbits which succumbed as compared with those serving as controls or with those which received normal serum.

Results of virus injections made one day after serum injection of the following doses.

Normal 1053...	3	0	3	0	3	0	3	0	12	0
----------------	---	---	---	---	---	---	---	---	----	---

[illegible]

TABLE 36.

Length of surviving periods of animals which died on account of virus injections on the day following serum treatment.

[illegible]

The 25 rabbits of the above series which survived the first virus injections were again injected with virus some two weeks later. Only two of the lot survived the second injection, and in the ones which succumbed the surviving periods were not materially longer than those shown by the control animals. The results of the second virus injections are shown in Tables 37-38 and they tend to show that the inoculation of a fatal amount of potent virus in a serum-protected animal is not followed by an active immunity of the same.

TABLE 37.

Results of second virus injections in serum-virus treated rabbits.

Serum No.	15 c.c.		10 c.c.		5 c.c.		2.5 c.c.		All doses	
	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
1053	3	0	3	0	2	0	3	0	11	0
1433	—	—	1	0	—	—	—	—	1	0
1436	1	0	2	0	1	0	—	—	4	0
1945	—	—	—	—	0	1	—	—	0	1
1946	2	0	2	1	3	0	—	—	7	1
Totals	6	0	8	1	6	1	3	0	23	2

[illegible]

TABLE 38.

Length of surviving periods of animals which were serum-virus treated and which died as a result of a second virus injection.

Serum No.	Approximate averages of surviving periods by doses and in hours							
	15 c.c.		10 c.c.		5 c.c.		All doses	
	No. of rabbits	Surviving periods	No. of rabbits	Surviving periods	No. of rabbits	Surviving periods	No. of rabbits	Surviving periods
1053.....	3	32	3	32	2	24	8	30
1433.....	—	—	1	24	—	—	1	24
1436.....	1	24	2	24	1	24	4	24
1946.....	2	24	2	24	3	24	7	24
Totals and averages..	6	28	8	27	6	24	20	26.4
Virus controls	—	—	—	—	—	—	8	24

In the second part of the experiment with the swine strain sera, all experimental animals received a uniform amount (10 c.c.) of serum and were then divided into groups of which each received a virulent inoculation at periods some three or four days apart. The details of the tests are exhibited in Tables 39-44.

1921

TABLE 39.

No. 1053

Rabbit No.	Injection of serum No. 1053, March 17, c.c.	Injection of virus No. 59, 1/5,000 c.c. Dates	Dates of deaths	Remarks
1	10	March 18	April 8	Death due to injection of 3/10,000 c.c. virus No. 59 on April 7.
2	10	March 18	April 8	Same as above.
3	—	March 18	March 19	Control.
4	—	March 18	March 21	Control.
5	10	March 21	April 9	Death due to injection of 1/5,000 c.c. virus No. 59 on April 7.
6	10	March 21	April 9	Same as above.
7	—	March 21	March 22	Control.
8	—	March 21	March 22	Control.
9	10	March 25	March 27	B. bipolaris in blood.
10	10	March 25	March 29	Many B. bipolaris in blood.
11	—	March 25	March 27	Control.
12	—	March 25	March 26	Control.
13	10	March 28	March 31	B. bipolaris in blood.
14	10	March 28	March 30	Blood swarming with B. bipolaris.
15	—	March 28	March 29	Control.
16	—	March 28	March 29	Control.
17	10	March 31	April 2	Blood swarming with B. bipolaris.
18	10	March 31	April 2	Few B. bipolaris in blood.
19	—	March 31	April 2	Control.
20	—	March 31	April 1	Control.

1921

TABLE 40.

No. 1172

Rabbit No.	Injection of serum No. 1172, April 6, c.c.	Injection of virus No. 59, April 17, 1/5,000 c.c.	Injection of virus No. 59, April 23, 1/6,000 c.c.	Dates of deaths	Remarks
1	10	+	+	April 24	Many B. bipolaris in blood.
2	10	+	+	April 24	
3	10	+	+	April 24	
4	10	+	+	April 24	
5	10	+	—	April 15	
6	10	+	+	April 24	Control first test. Control first test. Control second test. Control second test.
7	—	+	—	April 9	
8	—	+	—	April 9	
9	—	—	+	April 24	
10	—	—	+	April 24	

TABLE 41.

1921

No. 1433

Rabbit No.	Injection of serum No. 1433, Aug. 26, 10 c.c.	Injections of virus No. 59, 1/5,000 c.c.					Dates of deaths	Remarks
		8/31	9/3	9/6	9/9	9/19		
1	+	+	—	—	—	+	Sept. 20	
2	+	+	—	—	—	—	Sept. 9	
3	+	+	—	—	—	—	Sept. 3	
4	—	+	—	—	—	+	Sept. 20	
5	—	+	—	—	—	—	Sept. 1	
6	+	—	+	—	—	+	Sept. 20	
7	+	—	+	—	—	+	Sept. 20	
8	+	—	+	—	—	+	Sept. 20	
9	—	—	+	—	—	+	Sept. 20	
10	—	—	+	—	—	+	Sept. 20	
11	+	—	—	+	—	—	Sept. 7	
12	+	—	—	+	—	—	Sept. 7	
13	+	—	—	+	—	—	Sept. 7	
14	—	—	—	+	—	—	Sept. 8	
15	—	—	—	+	—	—	Sept. 7	
16	+	—	—	—	+	—	Sept. 10	
17	+	—	—	—	+	—	Sept. 10	
18	+	—	—	—	+	—	Sept. 10	
19	—	—	—	—	+	—	Sept. 12	
20	—	—	—	—	+	+	Lived.

TABLE 42.

1921

No. 1436

Rabbit No.	Injection of serum No. 1436, Sept. 9, 10 c.c.	Injection of virus No. 59, 1/5,000 c.c.				Dates of deaths	Remarks
		9/12	9/15	9/19	9/22		
1	+	+	—	—	—	Sept. 16	
2	+	+	—	—	—	Sept. 17	
3	+	+	—	—	—	Sept. 17	
4	—	+	—	—	—	Sept. 13	
5	—	+	—	—	—	Sept. 13	
6	+	—	+	—	—	Sept. 20	
7	+	—	+	—	—	Sept. 20	
8	+	—	+	—	—	Sept. 17	
9	—	—	+	—	—	Sept. 23	
10	—	—	+	—	—	Sept. 17	
11	+	—	—	+	—	Sept. 20	
12	+	—	—	+	—	Sept. 20	
13	+	—	—	+	—	Sept. 20	
14	—	—	—	+	—	Sept. 21	
15	—	—	—	+	—	Sept. 20	
16	+	—	—	—	+	Sept. 23	
17	+	—	—	—	+	Sept. 23	
18	+	—	—	—	+	Sept. 23	
19	—	—	—	—	+	Sept. 23	
20	—	—	—	—	+	Sept. 23	

TABLE 43.

1922

No. 1945

Rabbit No.	Injection of serum No. 1945, March 13, 10 c.c.	Injections of virus No. 59, 1/10,000 c.c.							Dates of deaths	Remarks
		3/17	3/21	3/25	3/29	3/31	4/4	4/8		
1	+	+	—	—	—	—	—	—	Mar. 18	
2	+	+	—	—	—	—	—	—	Mar. 19	
3	+	+	—	—	—	—	—	—	Mar. 18	
4	—	+	—	—	—	—	—	—	Mar. 18	
5	—	+	—	—	—	+	—	—	April 1	
6	+	—	+	—	—	—	—	—	Mar. 22	
7	+	—	+	—	—	—	—	—	Mar. 22	
8	+	—	+	—	—	—	—	—	Mar. 22	
9	—	—	+	—	—	—	—	—	Mar. 22	
10	—	—	+	—	—	—	—	—	Mar. 22	
11	+	—	—	+	—	—	—	—	Mar. 26	
12	+	—	—	+	—	—	—	+	April 9	
13	+	—	—	+	—	—	—	—	Mar. 26	
14	—	—	—	+	—	—	—	+	April 9	
15	—	—	—	+	—	—	—	—	Mar. 27	
16	+	—	—	—	+	—	—	—	Mar. 30	
17	+	—	—	—	+	—	—	—	Mar. 30	
18	+	—	—	—	+	—	—	—	Mar. 30	
19	—	—	—	—	+	—	—	—	Mar. 30	
20	—	—	—	—	+	—	—	—	Mar. 30	
21	—	—	—	—	—	+	—	—	April 3	
22	—	—	—	—	—	+	—	—	April 1	
23	—	—	—	—	—	—	+	—	—	
24	—	—	—	—	—	—	+	—	April 11	
25	—	—	—	—	—	—	—	+	April 9	
26	—	—	—	—	—	—	—	+	April 9	

TABLE 44.

1922

No. 1946

Rabbit No.	Injection of serum No. 1946, March 13, 10 c.c.	Injections of virus No. 59, 1/10,000 c.c.							Dates of deaths	Remarks
		3/17	3/21	3/25	3/29	3/31	4/4	4/8		
1	+	+	—	—	—	+	—	—	April 1	
2	+	+	—	—	—	+	—	—	April 1	
3	+	+	—	—	—	+	—	—	April 1	
4	+	+	—	—	—	—	—	—	Mar. 18	
5	—	+	—	—	—	—	—	—	Mar. 19	
6	+	—	+	—	—	—	—	—	Mar. 22	
7	+	—	+	—	—	—	+	—	
8	+	—	+	—	—	—	—	—	Mar. 23	
9	—	—	+	—	—	—	—	—	Mar. 22	
10	—	—	+	—	—	—	—	—	Mar. 22	
11	+	—	—	+	—	—	—	—	Mar. 26	
12	+	—	—	+	—	—	—	—	Mar. 26	
13	+	—	—	+	—	—	—	—	Mar. 26	
14	—	—	—	+	—	—	—	+	April 9	
15	—	—	—	+	—	—	—	—	Mar. 26	
16	+	—	—	—	+	—	—	—	Mar. 30	
17	+	—	—	—	+	—	—	—	Mar. 30	
18	+	—	—	—	+	—	—	—	Mar. 30	
19	—	—	—	—	+	—	—	—	Mar. 30	
20	—	—	—	—	+	—	—	—	Mar. 30	
21	—	—	—	—	—	+	—	—	Mar. 31	
22	—	—	—	—	—	+	—	—	April 1	
23	—	—	—	—	—	—	+	—	
24	—	—	—	—	—	—	+	—	April 11	
25	—	—	—	—	—	—	—	+	April 9	
26	—	—	—	—	—	—	—	+	April 9	

The general results of the tests exhibited in the preceding tables are shown in Tables 45 and 46.

It is manifest that in some of the sera examined a striking protective quality is present, but it is also manifest that even in the most potent specimens the passive immunity conferred is of but short duration and that as a rule it has vanished within a week. The influence of the serum is further shown by the lengths of the surviving periods of the animals. It will be observed that as the intervals between serum and virus inoculations lengthen there is a corresponding shortening of the surviving periods. The behavior of the surviving periods is further shown by the graph exhibited in Table 47.

TABLE 45.

Results of virus inoculations of serum-treated rabbits after varying periods.

Injection of 10 c.c. of serum No.	Virus injection after serum. Days	Serum-treated rabbits		Virus-control rabbits		Remarks
		Alive	Dead	Alive	Dead	
1053.....	1	2	0	0	2	
	4	2	0	0	2	
	8	0	2	0	2	
	11	0	2	0	2	
	14	0	2	0	2	
1172.....	1	5	1	0	2	
	5	1	2	1	1	
1433.....	8	3	0	2	0	
	11	0	3	0	2	
	14	0	3	1	1	
	3	0	3	0	2	
1436.....	6	0	3	0	2	
	10	0	3	0	2	
	13	0	3	0	2	
	4	0	3	1	1	
1945.....	8	0	3	0	2	
	12	1	2	1	1	
	16	0	3	0	2	
	4	3	0	0	2	
1946.....	8	1	2	0	2	
	12	0	3	1	1	
	16	0	3	0	2	

TABLE 46.

The average surviving periods, in hours, of rabbits dead as the result of virus injections at varying periods after serum treatment.

Injection of 10 c.c. of serum No.	Virus injection after serum. Days	Serum-treated rabbits		Virus-control rabbits		Remarks
		No. of rabbits	Surviving periods. Hours	No. of rabbits	Surviving periods. Hours	
1053.....	1	—	—	2	48	
	4	—	—	2	24	
	8	2	72	2	36	
	11	2	60	2	24	
1172.....	14	2	48	2	36	
	1	1	192	2	48	
	5	2	144	1	24	
1433.....	8	—	—	—	—	
	11	3	24	2	36	
	14	3	24	1	72	
1436.....	3	3	112	2	24	
	6	3	96	2	120	
	10	3	24	2	36	
	13	3	24	2	24	
	4	3	32	1	24	
1945.....	8	3	24	2	24	
	12	2	24	1	48	
	16	3	24	2	24	
	4	—	—	2	36	
1946.....	8	2	36	2	24	
	12	3	24	1	24	
	16	3	24	2	24	

TABLE 47.

Differences, in hours, between surviving periods of serum-treated rabbits and of corresponding virus-control rabbits at varying periods after serum treatment.

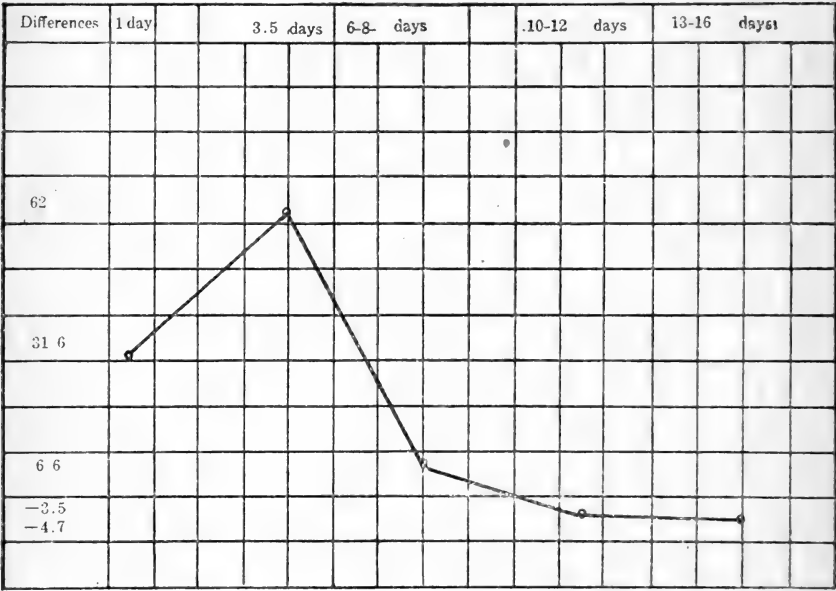


Table 48 shows the results of subsequent virus inoculations at varying periods after serum and first virus injections. It is shown that in all animals but one the second virus injections killed promptly and that the surviving periods were not materially different from those usually encountered in the virus controls.

TABLE 48.

Results of subsequent virus injections in serum-treated rabbits which survived previous virus inoculations.

Serum No.	1st virus injection in days after serum	2nd virus injection in days after first virus	Results		Surviving periods	Remarks
			Dead	Alive		
1053....	1	20	+	—	24	
	1	20	+	—	24	
	4	17	+	—	48	
	4	17	+	—	48	
1172....	1	16	+	—	24	
	1	16	+	—	24	
	1	16	+	—	24	
	1	16	+	—	24	
1433....	5	19	+	—	24	
	8	16	+	—	24	
	8	16	+	—	24	
	8	16	+	—	24	
1945....	12	14	+	—	24	
1946....	4	14	+	—	24	
	4	14	+	—	24	
	4	14	+	—	24	
	8	15	—	+	—	

SUMMARY OF RESULTS

1. Of the sera against hemorrhagic septicemia of cattle and swine examined, some show marked protective qualities which are expressed (a) by preventing death in a considerable number of the experimental animals when the latter were inoculated with virulent cultures and (b) by a lengthening in the surviving periods of those which succumbed to the infection. In other sera examined, the potency was not sufficient to prevent the death of a considerable number of the animals injected and the protective qualities of the serum were only shown by a more or less manifest lengthening of the surviving periods.

2. However marked the protective qualities of a given serum may be, the passive immunity conferred by it is of a rapidly evanescent character and completely vanishes within the space of one week.

3. Animals treated with serum and virulent culture do not become actively immune; in other words, the virus injected into a passively protected animal in no way renders the immunity more lasting. Animals so treated uniformly succumbed to a subsequent injection of virus.

4. The fact that certain sera are in a measure protective against infection by *Bacillus bipolaris septicus* warrants the hope that it may be possible so to improve them as to cause them to be of practical value. Further study and investigation with that object in view are both needed and justifiable.

(4M)

